

**Journal of Endodontics, 1996, Vol. 22**

**JULY**

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## **The disodium salt of EDTA inhibits the binding of vasoactive intestinal peptide to macrophage membranes: endodontic implications**

*Segura JJ, Calvo JR, Guerrero JM, Sampedro C, Jimenez A, Llamas R. The disodium salt of EDTA inhibits the binding of vasoactive intestinal peptide to macrophage membranes: endodontic implications. J Endodon 1996;22:337-40.*

**PURPOSE:** To investigate the effect of EDTA on vasoactive intestinal peptide (VIP) binding to macrophage membranes (MM's).

**M&M:** Binding assays were conducted on macrophage membranes at 15<sup>0</sup> C in 0.5 ml of 50 mM Tris-HCl buffer (pH 7.5) containing 1.6% (w/v) bovine serum albumin, 1.2 mg/ml of bacitracin, and different EDTA concentrations, using 45 pM of [<sup>125</sup>] VIP as tracer.

**RESULTS:** EDTA inhibited VIP binding to macrophage membranes in a dose-dependent manner. Low EDTA concentrations showed a light, but not significant, inhibitory effect on VIP binding. 5 mM of EDTA (a lower concentration than used in endodontics) caused a 55% inhibition of tracer binding to membranes, and EDTA concentrations of 10 mM inhibited binding by 72%. High EDTA concentrations (equal or higher than 100 mM) abolished binding. The concentration of EDTA that caused half-maximal inhibition (IC<sub>50</sub>) was 5.4 mM.

**C&C:** VIP is considered a neuroimmunoregulatory peptide involved in the modulation of lymphocyte and macrophage functions. VIP immunoreactive fibers have been observed in the paradentinoblastic region, within the nerve plexus of Raschkow, in the vicinity of blood vessels. The results of this experiment indicate that apical extrusion of EDTA could modify VIP-macrophage interaction modulating the inflammatory mechanisms involved in periapical lesions.

**July 1996**

**Orest M. Harkacz, Sr.**

## **Torsional Properties of Stainless Steel and Nickel-Titanium Endodontic Files**

*Rowan MB, Nicholls JJ, Steiner J. Torsional Properties of Stainless Steel and Nickel-Titanium Endodontic Files. J Endodon 1996;22:341-5.*

**Purpose:** To compare NiTi files with traditional SS K-type files under torsional loading, and to measure file diameters at 1 mm & 16 mm from the tip & compare with ANSI/ADA specification # 28.

**M&M:** Flex-O-Files & K-type files, sizes 15, 25, 35, 45, & 55 were compared. Torque at yield, torque at failure, rotation at yield, and rotation at failure were measured when files were rotated in a clockwise (CW) and counterclockwise (CCW) direction. File diameters at 1 mm & 16 mm from the tip were measured with vernier calipers.

**Results:** At the 1 mm level, all but sizes 15 of the SS & NiTi groups and sizes 45 & 55 of the NiTi group were significantly smaller than ANSI/ADA requirements. CW rotation at failure was significantly greater for the SS than the NiTi, except size 15. CCW rotation at failure was greater for the NiTi files in all sizes. There were essentially no differences in the amount of torque it took to cause failure in both the CW and CCW directions. The direction to failure was different, but the actual force it took to cause failure was the same. Only brittle fractures were observed (smooth-ended).

**C&C:** In CW rotation to failure, the files must first unwind, then reverse wind, then fracture. In CCW rotation, the files wind tighter, then fracture - the rotations are less because the files don't unwind first, and failure occurs much sooner. The strength and flexibility of NiTi give it a small advantage in CCW rotation, but not much.

**July 1996**

**Robin E. Hinrichs**

## **Production of Interleukin-1 by polymorphonuclear leukocytes resident in periradicular tissue**

*Miller GA, DeMayo T, Hutter JW. Production of Interleukin-1 by polymorphonuclear leukocytes resident in periradicular tissue. J Endodon 1996;22:346-51.*

**PURPOSE:** To determine whether polymorphonuclear leukocytes (PMN's) present in human periradicular lesions produce interleukin (IL)-1 $\alpha$  and IL-1 $\beta$ .

**M&M:** Fresh samples obtained from 21 patients undergoing endodontic root end surgery were prepared for light microscopic studies and tissue culture. Selected patients were not currently receiving long-term anti-inflammatory medications. Each of the periradicular lesions was at least 4 mm in diameter. Those tissue samples showing significant numbers of PMN infiltration were prepared for immunoperoxidase identification of IL-1 $\alpha$  and IL-1 $\beta$ -producing cells using specific polyclonal antibodies. Cell suspensions from selected periapical granuloma specimens, as well as from purified peripheral blood PMN's and peripheral blood mononuclear cells, were subjected to IL-1 quantitation using a commercial ELISA procedure.

**RESULTS:** In selected tissue specimens, 90% or more of the PMN's were found to stain positively for IL-1 $\alpha$  and IL-1 $\beta$ . In addition, significant numbers of plasma cells and tissue histiocytes stained positively for these IL's. The cells subjected to IL-1 quantitation using the ELISA procedure were found to produce significant levels of IL and could be stimulated to produce increased levels after coculture with lipopolysaccharide.

**C&C:** PMN's were (until recently) thought to be important only in the efferent limb of the immune response and were regarded as terminally differentiated cells incapable of protein synthesis. These results suggest that PMN's in inflammatory periradicular tissues may be a significant source of IL-1, and their possible roles in the establishment and resolution of periradicular lesion need to be re-evaluated.

**July 1996**

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## Effect of Protoporphyrin IX Limitation on Porphyromonas gingivalis

*Schifferle RE, Shostad SA, Bayers-Thering MT, Dyer DW, Neiders M. Effect of Protoporphyrin IX Limitation on Porphyromonas gingivalis. J Endodon 1996;22:352-55.*

**Purpose:** To evaluate the effect of protoporphyrin IX (the iron-free precursor of heme) limitation on *P. gingivalis* proteolytic enzyme production, virulence in a mouse abscess model of tissue invasiveness, and cell membrane protein expression.

**M&M:** *P. gingivalis* was collected from a perio patient. Bacterial cells were cultured in a protoporphyrin IX (P-IX) restricted medium, then in cultures with varying amounts added. Proteolytic enzyme assays were accomplished with cells grown in different concentrations of P-IX. The different concentrations were injected into mice subcutaneously, and the mice were killed in 24h intervals. Cells membranes were examined with electrophoresis.

**Results:** In the absence of heme, P-IX is essential for growth of *P. gingivalis*. A concentration of 0.25  $\mu\text{M}$  P-IX produced < 50% growth when compared with cells grown with P-IX excess. There was no difference between percentage of growth inhibition and the enzyme activity produced. There was no difference in survival and clinical appearance between mice injected with organisms grown with or without P-IX. P-IX deficiency inhibited growth to 73%, & resulted in a loss of membrane proteins at known sites, & the appearance of new membrane proteins.

**C&C:** So, this P-IX seems to be needed for *P. gingivalis* growth. If its not there, they don't grow as well. But the ones that do grow have altered cell membranes ( they adapt to their environment) and they still produce all their proteolytic enzymes. This seems to be a perio article denied publication, so they added a few sentences in the intro. and at the end of the discussion to try & relate it to endodontics. The bottom line is they were trying to manipulate the environment of these bacteria to decrease or eliminate their growth & virulent effects.

July 1996

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## Antibacterial activity of 10% carbamide peroxide bleaching agents

*Gurgan S, Bolay S, Alaçam R. Antibacterial activity of 10% carbamide peroxide bleaching agents. J Endodon 1996;22:356-7.*

**PURPOSE:** To examine the antibacterial effect of three commercial 10% carbamide peroxide bleaching agents against certain oral bacteria.

**M&M:** The antibacterial activity of three commercially available 10% carbamide peroxide bleaching agents (Nite White, Karisma, and Opalescence) on *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus sanguis*, *Lactobacillus casei*, and *Lactobacillus acidophilus* was examined. A 0.2% chlorhexidine solution was included as a positive control. The materials were inserted in wells punched on the surface of disk sensitivity agars seeded with the bacteria, incubated for 24 to 48 hours, and measured for zones of inhibition.

**RESULTS:** All three bleaching materials demonstrated higher antibacterial effect than the 0.2% chlorhexidine solution. Nite White showed the largest inhibition zone for *S. mitis*, and Karisma showed the same zone for *S. mutans* and *L. casei*. Opalescence had a pH value of 6.60, Nite White had a pH value of 5.26, and Karisma had a pH value of 5.16.

**C&C:** The materials with the lower pH had higher zones of inhibition, though no statistical analysis was performed to determine if this was significant. Since they all contain 10% carbamide peroxide as the active ingredient, the pH appears to be the variable responsible for the noted differences.

July 1996

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## **Regulation of Pulp Cell Matrix Metalloproteinase Production by Cytokines and Lipopolysaccharides**

*Panagakos FS, O'Boskey JF, Rodriguez E. Regulation of Pulp Cell Matrix Metalloproteinase Production by Cytokines and Lipopolysaccharides. J Endodon 1996;22:358-61.*

**Purpose:** To determine the effects of inflammatory cytokines (IL-1 $\alpha$ , IL-1 $\beta$ , & TNF- $\alpha$ ) and lipopolysaccharides (LPS's) on the production and secretion of matrix metalloproteinases (MMP's) by primary human and clonal rat pulp cells *in vitro*.

**M&M:** Human pulp cell cultures were established. Human and rat pulp cells were plated & treated with appropriate concentrations of LP's or cytokines. Trypsin was used to activate the MMP enzymes, and soybean trypsin inhibitor was used to inhibit the trypsin after 15 min. The MMP activities were assayed using electrophoreses and stains.

**Results:** In unactivated samples, IL-1 $\beta$  and TNF- $\alpha$  stimulated secretion of MMP's. IL-1 $\alpha$  seemed to have no effect. When activated with trypsin, all 3 cytokines induced higher levels of MMP's. IL-1 $\alpha$  may stimulate secretion of MMP, but does not seem to be involved with the activation of the enzymes after secretion. Similar results were obtained with both rat & human pulp cell lines. However, different MMP's were affected.

**C&C:** The authors were theorizing that accumulation of bacterial products in the pulp could stimulate macrophages to produce cytokines, which could inhibit differentiation of pulp cells into odontoblasts and stimulate the production of matrix degrading enzymes. If we could alter the action of the inflammatory cytokines on the pulp, we might better aid the pulp when assaulted with caries.

**July 1996**

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## **Intracanal pH changes of calcium hydroxide pastes exposed to carbon dioxide in vitro**

*Fuss Z, Rafaeloff R, Tagger M, Szajkis S. Intracanal pH changes of calcium hydroxide pastes exposed to carbon dioxide in vitro. J Endodon 1996;22:362-4.*

**PURPOSE:** To assess the effect of exposure for 30 days to CO<sub>2</sub> on the pH of several calcium hydroxide pastes within the root canal, in vitro.

**M&M:** Sixty-two extracted young permanent, single-rooted human teeth were used in the study. Cementum was removed from the buccal and palatal surfaces to expose dentinal tubules. The teeth were endodontically prepared up to a size 60 using K-files (apical patency was maintained with a size 25 file). After instrumentation, the ground external root surface of the dentinal walls of the root canals were etched with an acid gel to ensure patency of the exposed dentinal tubules. The teeth were randomly divided into six experimental groups of 10 teeth each to be filled with either Calxyl, Hydrocalcium, or a paste made by mixing calcium hydroxide powder with either distilled water, camphorated p-monochlorophenol, local anesthetic solution, or Solvidont. The controls included two prepared but unfilled teeth submerged in vials containing 10 mL of distilled water; 12 vials (two for each experimental material) in which 0.022 g of calcium hydroxide paste mixed with water was added to 4 mL water and four vials with 4 mL medium only. Access cavities were sealed with Cavidentin, and the teeth were placed individually in vials containing 10 mL distilled water. Five vials of each group were exposed to air at room temperature, whereas the other five vials were exposed to carbon dioxide in a closed container. The pH of the paste in the root canals was measured after 30 days.

**RESULTS:** The initial pH of all the calcium hydroxide preparations ranged from 13 to 13.2; the initial pH of the surrounding medium in the vials (distilled water) was 7.4. Materials recovered from the root canal of the teeth exposed to air maintained their high pH during the experimental period (mean 13.1), whereas those sealed in teeth exposed to CO<sub>2</sub> showed a significant reduction in pH (mean 12.54). The pH of the medium surrounding the teeth in the vials that were exposed to air rose from 7.4 to a mean of 8.47, whereas the pH of the medium surrounding the teeth exposed to CO<sub>2</sub> was reduced from 7.4 to a mean of 6.65. The pH of the pastes in the six control vials exposed to air did not change at the end of 30 days, but a significant reduction was observed in the pH of the pastes in the six control vials exposed to CO<sub>2</sub> (mean 7.53). The pH of the distilled water in the control vials containing medium only and the vials containing two unfilled teeth did not change when exposed to air, but was lowered to a mean of 4.6 when exposed to CO<sub>2</sub>.

**C&C:** In this experiment, the material was subjected to 100% CO<sub>2</sub> in air tight containers, which is not the concentration or condition of CO<sub>2</sub> normally found within the tissues. To more accurately approximate the clinical conditions in which a calcium hydroxide paste would be used, the experiment should have used the gas mixtures normally found in human tissues

**July 1996**

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## **An In Vitro evaluation of Microleakage of a New Root Canal Sealer**

*Rohde TR, Bramwell JD, Hutter JW, Roahen JO. An In Vitro evaluation of Microleakage of a New Root Canal Sealer. J Endodon 1996;22:365-7.*

**Purpose:** To compare the apical microleakage of Ketac-Endo to Roth's 801 elite and AH26, and to compare Ketac-Endo used with a single cone technique to a lateral condensation technique.

**M&M:** 64 teeth were decoronated, instrumented, and randomly assigned to one of 4 groups. 4 teeth served as controls. Roots were condensed with lateral condensation & Ketac-Endo, Roth's elite, or AH26, and with Ketac-Endo & a single cone technique. After setting for 8d, apical leakage was determined by suspending the apices in methylene blue for 6 days. The teeth were split & viewed under a stereomicroscope. Leakage was divided into 3 ranges < 0-3, > 3-6, and >6 mm.

**Results:** Roth's 801 elite was significantly better than both Ketac-Endo groups. AH26 was significantly better than Ketac-Endo using lateral condensation. No significant differences were found between Roth's 801 & Ketac-Endo laterally condensed. With the exception of 2 roots, the both Ketac-Endo groups showed complete dye penetration. Possibly the sealer had not set. It also seemed that the Ketac-Endo sealer absorbed the dye, not just permitted leakage at the sealer interfaces. It may be that Ketac-Endo may absorb bacteria also. It is noteworthy that all of the groups had significant leakage at all levels measured.

**C&C:** If you like Ketac-Endo, this study would support a single cone technique when compared to lateral condensation. The authors don't mention any problems with quick setting time for the Ketac-Endo with lateral condensation group - I wonder how they accomplished it.

**July 1996**

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## **Comparison of nickel-titanium and stainless steel hand-file instrumentation using computed tomography**

*Gambill, JM, Alder M, del Rio CE. Comparison of nickel-titanium and stainless steel hand-file instrumentation using computed tomography. J Endodon 1996;22:369-75.*

**PURPOSE:** To evaluate standard design Ni-Ti hand files as compared with stainless steel files using filing and reaming instrumentation techniques; and introduce and evaluate a CT image analysis system in comparing endodontic instrumentation.

**M&M:** Thirty-six single-rooted teeth of similar shape and canal size were divided into three groups. In group A, canals were instrumented using a quarter turn/pull technique with K-flex files. In group B, canals were prepared with Ni-Ti hand files (Mity files) using the same technique as group A. Group C was prepared with Ni-Ti hand files (Mity files) using a reaming technique. Group D consisted of four teeth that were not instrumented. The teeth were scanned by computed tomography before and after instrumentation. Reformatted images of the uninstrumented canals were compared with images of the instrumented canals.

**RESULTS:** There were no significant differences in root canal curvature among the three instrumented groups. The mean uninstrumented canal curvature was 18.8 degrees in the mesial-distal plane. Group C (Ni-Ti instruments used in a reaming technique) caused significantly less canal transportation, removed significantly less volume of dentin, required less instrumentation time, and produced more centered and rounder canal preparations than K-flex stainless steel or Ni-Ti files used in a quarter turn/pull technique.

**C&C:** The authors noted that in wide or irregular-shaped canals, the reaming technique produced round preparations in the center or at one side of the canal space and left the remaining canal walls uninstrumented in the coronal two-thirds of the canal (although the apical third appeared to be adequately instrumented). The use of combination techniques (such as reaming in conjunction with filing) may be more appropriate for instrumenting canals to overcome this problem.

**July 1996**

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## **Surgical Repositioning of Unerupted Anterior Teeth**

*Saad AY, Abdellatief EM. Surgical Repositioning of Unerupted Anterior Teeth. J Endodon 1996;22:376-9.*

**Purpose:** To evaluate the condition of the root & supporting tissues of unerupted anterior teeth kept in situ and gradually repositioned to the occlusal plane.

**M&M:** 12 patients, with impacted anteriors with complete root formation were included. After removal of the causes of noneruption, the teeth were surgically exposed, repositioned with elevation (without complete removal), and splinted in place. After 2 weeks, a pulpectomy was accomplished, and 1 week later the RCT was completed and the splint removed. No medicaments were used in the canal. Patients received broad-spectrum antibiotics 1 day prior to the surgery, and for 4 days after. Patients were followed for 6 months to 3.5 years to evaluate success.

**Results:** No noticeable complications, rejection, infection or ankylosis occurred. Root resorption with adjacent radiolucency was not observed, and periodontal ligaments were established and intact.

**C&C:** The obvious comparison of this technique is to a luxation injury with no exarticulation. If care is taken, the PDL will suffer less injury than in a trauma case, and the cells are never exposed to the environment. Coverage with antibiotics was an attempt to prevent bacterial contamination during the surgical procedure. We would expect these good results, but the patients should still be monitored, for at least 5 years post surgery.

**July 1996**

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## Long-term, postsurgical results on teeth with periapical radiolucencies

*August DS. Long-term, postsurgical results on teeth with periapical radiolucencies. J Endodon 1996;22:380-3.*

**PURPOSE:** To perform a long-term retrospective study of endodontic surgical procedures in which the author, trained as an endodontist, was the only operator.

**M&M:** Patients undergoing endodontic surgical procedures in which it was possible to take a biopsy made up the group for this study. The study was retrospective, evaluating surgical cases treated from September 1969 to December 1983 in order to have at least a 10-yr follow-up. All cases that received root-end fillings were filled with zinc-free amalgam. Forty-one teeth in 39 patients were able to be evaluated. These teeth had either an apicoectomy, an apicoectomy with a root-end amalgam, or in two cases, a root amputation.

**RESULTS:** Thirteen male and 26 female patients were recalled, with ages ranging from 20 to 64 years. The time period for recall following surgery ranged from 10.9 to 23.5 years, with the median recall being 15.2 years. Cases in which apicoectomy alone was performed had an 82.6% success rate. Cases that had a root-end filling placed in addition to the apicoectomy had a success rate of 62.5%.

**C&C:** During the time period noted in the M&M, 205 patients had 220 teeth biopsied. Of these, 27 teeth were known to have been extracted (six because of continuing periapical problems), but they were not included in the study. Only cases in which the teeth were still present were used in the study. Since the sample recalled was small, and the other cases were not included, the above values may not be an accurate reflection of the postsurgical results. Also, the results reflect the success rates for *this author only* with respect to *his* technique in root resection and root end filling. A study evaluating a large patient population from multiple practitioners would provide more reliable data regarding success rates attributed to the techniques.

July 1996

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